NO. 2

BIO-RESEARCH CONSULTANTS, INC.
9 Commercial Avenue
Cambridge, Massachusetts 02141

R E P O R T

to

Philip Morris Incorporated

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A. TECHNICAL DEVELOPMENTS

It was planned previously to study the effect of smoke and its gas phase on salivation in a dose-related fashion, with smoke concentration of 10, 20 and 30 percent. To perform these studies, attachments to the smoking machine were made to accommodate the simultaneous burning of one, two or three cigarettes. Since it is known that the filtering capacity of the Cambridge filter to cigarette smoke is not materially altered during a total of up to

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fifteen puffs (Dr. Bernfeld), it was decided to change the Cambridge filter after every five puffs, with the smoking of either one, two or three cigarettes.

B. SMOKE STUDIES

I. Introduction

The results of previous experiments established that the exposure to smoke inhibits the salivary performance of male randombred Millerton Swiss and inbred C57BL mice (Reports Nos. 17 and 19). It was further established that this salivary inhibition is much less marked when using the gas phase of tobacco smoke alone (Report No. 17). The sole smoke concentration applied in these studies was that of 10 percent, thus the results do not permit information on any quantitative dose-effect relationships.

A previous study also showed the feasibility of the salivation method for brand comparisons among cigarettes, but the experiment had been performed inefficiently and an improved experimental smoking procedure had been suggested (Report No. 19).

The present study was designed, on the one hand, to strengthen the results of earlier experiments and to explore new aspects of cigarette smoke on salivation in mice, on the other hand, all in one large study.

The specific objectives of the study are:

(1) to confirm or disprove the inhibitory action of whole tobacco smokes on salivary performance of mice in a dose-related fashion;

(2) to examine the inhibitory action of the gas phase of tobacco smokes on salivary performance of mice in a dose-related fashion;

(3) to establish any relationships between the biological effects from whole smokes and from their gas phases;

(4) to define the biological effects from each of four brands of cigarettes and to examine any differences among them.

II. Experimental

This study examines the salivary inhibition of mice by three concentrations of whole smoke and their corresponding gas phases in four brands of cigarettes.

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Male C57BL mice, approximately 24 weeks old, which had been housed for some time six to a cage while wearing collar, were used. The mice had been adapted previously to the salivation procedure and were all in good health. In any smoking experiment, salivary performance of mice was determined four times with a one-hour interval between measurements. The first and second measurements served to establish the control salivary performance of individual mice during that particular day. A ten-minute smoke treatment was applied immediately prior to the third measurement. The fourth measurement was performed to examine any aftereffects to treatments. During the time intervals between any two salivation measurements the mice were returned to their "treatment" cages which were supplied with adequate food and water. Salivary performance in mice was determined according to earlier described standard procedures. During the course of this study, some mice were re-used. This was done only after allowance of at least one week of rest to eliminate possible aftereffects from earlier experiments.

The conditions in the smoking machine were standardized to 35 ml puffs of two seconds' duration taken at intervals of 58 seconds. The smoke would persist for 15 seconds in the inhalation chamber and then be expulsed in three seconds, followed by fresh air purge for 40 seconds. Unless specified otherwise, ten puffs were studied in each case, using the first five puffs of each of two sets of cigarettes smoked without interruption in the on-puff-per-minute cycle.

In order to obtain 10, 20 or 30 percent smoke concentration, the smoking machine burned simultaneously one, two or three cigarettes. The purge pressure of the smoking machine had to be reset from 31 to 62 to 93 in order to double or triple the amounts of tobacco burned. Butt lengths of cigarettes were determined to examine the relative success of this procedure.

In order to obtain the gas phases from tobacco smokes, Cambridge filters were used. They were interposed into the smoke stream between the proximal end of the cigarette and the inhalation chamber. After every five puffs a new Cambridge filter was inserted in order to maintain the same filtering capacity of the filters, as described under A.

The experimental cigarettes were stored and equalized in length to 65 mm. They were climatized in a descicator for at least several days until immediately prior to their actual use in the smoking machine.

The various cigarettes brands examined were "Pall Mall," "ADG Burley," "ADF Bright" and "ABU TFP." As proper controls served "machine exposed only" and "no exposure."

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A single experiment started off with determining the first and second salivation measurements on 42 mice. The sum of these two measurements served to rankorder the mice according to salivary performance. The 36 highest salivating mice were assigned systematically to any one of six treatment groups (the four different tobaccos, "machine exposed" or "no exposure") and the remaining mice were eliminated from this experiment. Thus the third and fourth salivation measurements were limited to maximally 36 mice, six mice in each of six treatment groups. The experiment was run twice and the results of the two experiments were pooled into a set. A set of data is thus based on four measurements from 72 mice, 12 mice per treatment group. One set of data was obtained on each of three concentrations of whole smoke and on each of the three corresponding gas phases. The entire study provided approximately 1,728 salivation measurements. Statistical treatment of the data was performed, using established statistical techniques through the Wang computer capability.

III. Results and Discussion

a) Mortality

The mortality rate per 12 male C57BL mice when exposed to various concentrations of whole smoke or gas phase from various brands of cigarettes is given in Table 93 and a breakdown per experiment in Table 94.

The data show that exposure to whole smoke is very toxic, when expressed in terms of number of death through exposure. The toxicity of whole smoke is dose-related for each of the four cigarette brands. A ten-minute exposure to 10 percent whole smoke shows no mortality in any brand; some deaths occur in some of the brands with 20 percent smoke; exposure to 30 percent smoke causes high mortality in each of the brands.

The smoke toxicity varies with the brand of tobacco. Concentrations of 20 and 30 percent whole smokes from ADG Burley and Pall Mall are very toxic in contrast to whole smokes from ABU TFP and ADF Bright which are moderately toxic with 30 percent smoke concentration alone. The lethality data are too limited to prove the differences in toxicity among the whole smokes of the brands to be significant, but the high reproducibility of mortality trends in each experiment (as shown in Table 94) supports this contention.

Incomplete results on cigarette lengths burned in the various experiments are summarized in Table 95. The data show that differences in the amounts of tobacco burned with each concentration in each brand of cigarette

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are relatively small. This then would indicate that the toxicity of whole smokes from different brands of tobacco are due to chemical composition rather than quantity of the whole smokes.

In contrast to the results with whole smoke, the gas phase of any of the brands shows no mortality even at a concentration of 30 percent. This suggests that the gas phase is nontoxic when compared with whole smoke. It can further be noted that the Cambridge filter causes a reduction of the amount of tobacco burned when the same settings of the smoking machine are used.

b) Immediate effect upon salivation

Tables 96, 100, 102, 106, 110 and 112 present salivary performance data from mice when exposed to 10, 20 or 30 percent whole smoke or gas phase. Tables 97, 101, 103, 107, 111 and 113 present their analyses of variance, and Tables 98, 99, 104, 105, 108, 109 and 114 provide differences in salivary performance among the individual brands. In this report we will restrict ourselves and extract some major aspects from these basic tables. Tables 115 and 116 are two of such extracted summary tables. They present a summary of results on salivary performance by male C57BL mice immediately following a ten-minute exposure to 10, 20 or 30 percent whole smoke or their corresponding gas phase in four brands of cigarettes, expressed in absolute values of boundary displacement, and as percentage from controls. These data clearly show that whole smoke inhibits salivation. The inhibitory effect is noticeable in smokes of only two brands at a 10 percent smoke concentration, but shows itself in all four brands with concentrations of 20 and 30 percent. The inhibitory effect on salivation increases in a dose-related fashion up to 20 percent in each of the four brands but only in two out of four brands with smoke concentrations above the 20 percent.

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The data further show that the corresponding gas phases of the various whole smokes consistently inhibit salivary performance, but the inhibitory effect is usually less severe than with whole smokes. The inhibitory effect of the gas phases of each of the brands is dose-related from 0 through 20 percent smoke concentrations. Ten percent gas phase shows a slight nonsignificant salivary inhibition in each brand, whereas the salivary inhibitions are significant in each brand with a 20 percent concentration. The data with 30 percent gas phase show inconsistent results among brands. The gas phases of Pall Mall and ADG Burley decrease salivary performance in a dose-related fashion but this is not the case with ABU TFP and ADF Bright. This result, if significant, would indicate that the gas phase factors which

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govern salivary inhibition are chemically different between Pall Mall and ADG Burley versus ABU TFP and ADF Bright. Our data are not sufficient to establish this aspect with certainty.

A comparison between the whole smoke and gas phase data reveals that, on the whole, the salivation inhibitory effects are much more severe with whole smoke than its gas phase. This suggests that a major portion of the salivary inhibitory factors are contained in the particulate fraction rather than the gas phase of whole smoke.

It is interesting to note that 10 percent whole smoke of ABU TFP and Pall Mall does not show any salivary inhibition. In view of the fact that significant inhibitory effects are obtained with 10 percent whole smokes from ADF Bright and ADG Burley, and that consistent mild inhibitions were noted with 10 percent gas phases in each of the four brands, it can be speculated that the "no effect" should be interpreted to mean that it is the resultant of stimulatory and inhibitory salivation factors contained in the particulate phase of these two brands of cigarettes.

c) Aftereffects upon salivation

Tables 117 and 118 summarize the results on salivary performance of C57BL mice one hour following the ten-minute exposure to 10, 20 or 30 percent whole smoke or gas phase from the four brands of cigarettes, expressed in absolute values and as percentage from controls.

The data on whole smoke show significant inhibitory aftereffects. The inhibitions are of approximately the same size as those observed one hour earlier. In contrast, no significant inhibitory effects were found in the gas phases at any concentration of any brand (with the exception of ADF Bright at 20 percent concentration, which cannot be explained at the present time).

These results show that any salivary inhibition factors of the gas phase are very transient and that the salivary inhibition factors of whole smoke are long-lasting in nature. The results indicate the long-lasting salivary inhibitory factors to be contained in the particulate fraction. The chemical nature of the transient and long-lasting salivary inhibitory factors of whole smoke is unknown.

d) Biological characterization of cigarette brands

Under the previous sections the various brands were discussed indirectly, using one specific parameter of measurement. The results are

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summarized here to characterize each brand. Tables 119, 120, 121 and 122 present the results obtained. The tables show data on mortality and salivary performance, immediately and one hour after a ten-minute exposure to whole smoke or its corresponding gas phase of each brand of cigarettes.

The observed differences between the brands are the following: Acute toxicity of whole smoke is very high with Pall Mall and ADG Burley, but low with ABU TFP and ADF Bright cigarettes. The acute toxicity is associated with the particulate phase of whole smoke. Whole smokes from each brand caused salivary inhibitions which were found to be quantitatively much the same over a period of one hour. Two exceptions to the salivary inhibition were found, namely 10 percent whole smokes from Pall Mall and ABU TFP which showed "no effect."

It was suggested previously that the particulate phase of whole smokes in Pall Mall and ABU TFP contains salivation stimulatory factors (see under e.).

A suggestion was also made that the gas phase factors governing salivary inhibition are chemically different in Pall Mall and ADG Burley versus ABU TFP and ADF Bright (see under b.).

The above results show a preliminary characterization of some biological effects with individual brands of cigarettes. They suggest that different brands of tobacco can be characterized through biological assay if one uses effectively the salivation inhibitory aftereffect. This possibility is as yet unexplored.

e) Relationships among mortality, direct effects and aftereffects

These relationships among response parameters of measurement can be examined in a variety of ways. We will first examine to what degree these parameters can be related to the concentrations applied and will examine any specific other relationships subsequently.

It should be recalled that the study incorporates data from four different tobaccos, smoked at each of three concentrations. Thus one can expect maximally four dose-response curves for whole smoke and for gas phase with each response parameter. Table 123 summarizes results from Table 119, 120, 121 and 122:

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Table 123

Theoretical and Actual Frequencies in Obtaining Dose-Response Curves

Parameter	Condition	Theoretical	Actual
Mortality	Whole smoke	4	4
"	Gas phase	4	ND
Direct effect	Whole smoke	4	1
"	Gas phase	4	1
Aftereffect	Whole smoke	4	3
"	Gas phase	4	0

The table shows the maximal and actual frequencies at which a dose-related response was obtained. "Mortality" and "salivation aftereffect" were indicative for whole smoke concentrations with 100 and 75 percent respectively. The "direct salivation effect" is not a good indication for smoke concentration. The gas phase results are too limited for any conclusions at the applied concentrations.

The results further show (a) that acute mortality from whole smokes is relatively well predicted through "salivation aftereffect" measurements at lower smoke concentrations and (b) that the degree of immediate salivary inhibition from whole smokes is very well related to the degree of salivary inhibition one hour later.

The facts that "mortality" and "salivation aftereffects" are rather good indicators for whole smoke concentrations and that "mortality" is terminal to the animals, whereas "salivation aftereffect" is not, suggest the usefulness of "aftereffect" measurements to predict short-term toxicity by whole smoke of cigarettes.

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IV. Summary

A study was designed to examine toxicological and pharmacological effects of whole smoke and gas phase from four different brands of cigarettes. The parameters of measurement were (1) mortality, (2) salivation inhibition directly after exposure and (3) salivation inhibition one hour after exposure.

The gas phase of each of the four smokes gave no mortality and exerted only a transient salivation inhibition which was not noticeably dose-related.

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Whole smoke of each of the four cigarettes showed acute mortality in a dose-related fashion. In the surviving animals, salivary performance was much more permanent and the degree of salivary inhibition, measured one hour after exposure, was dose-related in a majority of cases. It was suggested that the degree of persistency of salivary inhibition with low whole smoke concentrations might be developed into a pharmacological method to predict acute toxicity of whole smoke at higher smoke concentrations. A preliminary biological characterization of each brand of tobacco was performed and the relative differences among them discussed.

C. NICOTINE STUDIES

I. Introduction

Earlier experiments studying the effect of nicotine tartrate on salivation have only provided limited, apparently contradictory results. High doses of subcutaneously injected nicotine tartrate to unselected randombred Millerton Swiss mice (with a low salivation level) stimulated salivation. However, lower doses of nicotine tartrate gave apparent inhibition (Report No. 18). A subsequent experiment with preselected high salivating randombred mice showed that 1 mg nicotine tartrate maintained salivary performance, whereas the lower doses seemed to inhibit salivation immediately after injection (Report No. 19).

It is the purpose of this experiment to study the salivary response of "genetically low" and "genetically high" salivating inbred mice to pharmacological doses of subcutaneously injected nicotine tartrate in a dose-related fashion.

II. Experimental

Male AKR/J and C57BL mice were obtained from the Jackson Laboratories in Bar Harbor, Maine, to serve as mice that salivate "genetically low" or "genetically high," respectively. The mice were housed six to a cage, collared and accustomed to the salivation procedure.

The size and layout of each single experiment with nicotine tartrate is identical to the experiments with smoke and include four salivation measurements on 36 mice, six mice to each of six treatment groups. The data of two such experiments were pooled into a set of data. Two sets of data were obtained in each strain.

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The six treatments were as follows: control, water, 0.03, 0.1, 0.3 and 1.0 mg nicotine tartrate injected subcutaneously immediately prior to the third salivation measurement. Salivation was measured according to established procedures. The results were analyzed similarly to the smoke data.

III. Results and Discussion

Table 124 presents the rate of mortality upon injections in AKR/J and C57BL mice. The data show that 1 mg nicotine tartrate is very toxic to AKR/J mice but that it remains a pharmacological dose for C57BL mice.

Tables 125 through 135 present summaries of salivary performances, their analyses of variance and analyses of differences among individual treatment means from AKR/J and C57BL mice.

C57BL mice - The mice included in the first experimental set were high salivators. The data show a nonsignificant trend for a salivation increase with the lowest nicotine treatment. The three higher nicotine doses significantly inhibited salivation immediately after injection. One hour later, a salivation inhibitory effect was noticeable with 0.3 and 1.0 mg nicotine tartrate.

The mice included in the second experimental set were the lower salivators. The data show a nonsignificant trend towards a stimulatory effect on salivation with each dose of nicotine tartrate. One hour later, the two highest doses of nicotine tartrate showed significant inhibitory aftereffects on salivation.

AKR/J mice - Both sets of data show (as was the case with the lower salivating C57BL mice) a trend towards an initial immediate stimulation of salivation upon injection with any dose of nicotine tartrate. This stimulatory effect is most pronounced with the lower levels of nicotine tartrate. The four doses of nicotine tartrate exerted inhibitory aftereffects on salivation which was found to be rather dose-related.

Although the above results are partly nonsignificant, we feel that they are real because these trends were consistent in each experiment within each set of data.

The above results tend to show that the immediate effect on salivation by nicotine tartrate can be either a stimulation or an inhibition. The type of response is not directly strain-dependent, but is among others determined by the salivation level of the animal and the dose used. Nicotine tartrate is

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mildly stimulatory to salivation in low-salivating mice immediately after injection over a broad range of doses, with largest stimulation of the lowest dosages. Nicotine tartrate exerts an immediate significant inhibitory effect on salivation in high-salivating mice, except when injected with low amounts of nicotine, when it could be slightly stimulatory. Nicotine tartrate has an inhibitory aftereffect on salivation with each strain, the inhibition is dose-related.

IV. Summary

A summary of the nicotine results is presented in table form - see Table 136.

D. RELATIONSHIPS BETWEEN SMOKE AND NICOTINE

Whole smoke consists of two fractions, the gas phase and the particulate phase. The inhibitory effects of gas phase of tobacco smoke was found to be very transient. The strong persistent inhibitory aftereffect on salivation was concluded to be due to the particulate phase of whole smoke. The particulate phase consists of a variety of chemical compounds which include nicotine. Thus the question whether the biological effects caused by the particulate phase can be explained through the pharmacological actions of nicotine, is appropriate.

The following own experimental evidence to date supports the above hypothesis:

1. Both whole smoke and nicotine exert an aftereffect on salivary performance, in which salivation is inhibited in a dose-related fashion over relatively long periods of time.

2. Nicotine tartrate will inhibit the immediate salivation of high salivating mice, except at very low nicotine concentrations. If the immediate pharmacological effects of whole smoke were those of nicotine, one should expect an immediate salivary inhibition through smoke, except with very low smoke concentrations. The observed immediate salivary inhibitions at high smoke concentrations and the apparent "no effects" with smokes from ABU TFP and Pall Mall at the lowest smoke concentration are in full agreement with the assumption.

In conclusion, the above discussion shows that the experimental evidence to date, on the basis of salivary results by smoke and nicotine, are compatible with the hypothesis that the effects of whole smoke could be accounted for by nicotine.

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E. CONCLUSIONS

1. The salivation methodology permits the biological characterization and preliminary differentiation among smokes from various brands of cigarettes.

2. The persistency of salivary inhibition with pharmacological smoke concentrations predicts reasonably well the acute toxicity of cigarette smoke at toxicological levels.

3. Nicotine exerts two types of immediate responses on salivation, namely inhibition or stimulation. The type of salivary response is independent of the strain of mice but is dictated, among other factors, by the base salivation level of the mice and the dose of nicotine employed.

4. Experimental evidence on smoke and nicotine to date supports the hypothesis that the pharmacological effect of whole smoke on salivation can be explained by the biological effects of nicotine.

5. The effect of gas phase of smoke upon salivation remains to be explained.

F. SUGGESTION FOR FURTHER RESEARCH IN SALIVATION

For the near future we plan to

(1) test the effect of whole smoke and gas phase on salivary performance of low-salivating AKR/J mice;

(2) test the effect of nicotine on salivary performance of low-salivating AKR/J mice;

(3) examine whole smoke and gas phase Sano with and without added nicotine on salivary performance of AKR/J and C57BL mice;

(4) develop salivation methodology into a method to predict quantitatively the acute toxicity of cigarette smoke without the loss of animals.

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Table 93

Rate of Mortality in Twelve Male C57BL Mice When Exposed to 10, 20 or 30 Percent Whole Smoke or Gas Phase from Various Kinds of Cigarettes (Cigarettes were all cut to 65 mm.)

Whole smoke

Treatments	Concentration			Total
	10	20	30	
Control	0	0	0	0
Machine exposed	0	0	0	0
ABU TFP	0	0	4	4
ADF Bright	0	1	2	3
ADG Burley	0	7	9	16
Pall Mall	0	4	12	16
Total	0	12	27	39

Gas phase

Treatments	Concentration			Total
	10	20	30	
Control	0	0	0	0
Machine exposed	0	0	0	0
ABU TFP	0	0	0	0
ADF Bright	0	0	0	0
ADG Burley	0	0	0	0
Pall Mall	0	0	0	0
Total	0	0	0	0

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Table 94

Rate of Mortality in Male C57BL Mice during Each of Two Experiments When Exposed to 10, 20 or 30 Percent of Whole Smoke

Whole smoke

Treatments	Concentration						Subtotal		Total
	10%		20%		30%				
	1	2	1	2	1	2	1	2	
Control	0	0	0	0	0	0	0	0	0
Machine exposed	0	0	0	0	0	0	0	0	0
ABU TFP	0	0	0	0	3	1	3	1	4
ADF Bright	0	0	0	1	0	2	0	3	3
ADG Burley	0	0	2	5	6	3	8	8	16
Pall Mall	0	0	1	3	6	6	7	9	16
Total	0	0	3	9	15	12	18	21	39

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Table 95

Mean Length of Cigarette Burned in First Five Puffs with Bio-Research Smoking Machine (expressed in millimeters)

Without Cambridge Filter

Cigarette Brand	Number of Cigarettes		
	1	2	3
Pall Mall	25.5*	ND	35.0†
ADG Burley	27.3	ND	34.0
ABU TFP	27.7	ND	35.0
ADF Bright	24.7	ND	30.0

* Mean of two measurements
† Mean of six measurements
ND Not determined

With Cambridge Filter

Cigarette Brand	Number of Cigarettes		
	1	2	3
Pall Mall	29.0*	34.5**	28.5†
ADG Burley	26.5	33.7	27.0
ABU TFP	30.5	34.0	31.5
ADF Bright	26.5	32.5	29.0

* Mean of two measurements
** Mean of four measurements
† Mean of six measurements

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Table 96

The Effect of Whole Smoke from Various Cigarettes on Salivary Performance of Male Inbred C57BL Mice
(Smoke concentration ten percent)

Treatment	Measurements			
	1	2	3	4
1 (control)	71.2*	65.5	76.4	81.5
2 (machine exposed)	80.1	58.8	49.8	75.0
3 (Pall Mall)	67.4	68.8	63.3	68.2
4 (ADG Burley)	71.0	69.0	30.7	55.7
5 (ABU TFP)	72.4	68.2	65.6	72.3
6 (ADF Bright)	63.2	78.1	33.0	48.6

* Mean salivary performance of 12 mice.

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Table 97

Analysis of Variance

Measurement 1

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	1,904	381	.53	2.37	3.34
Error	66	47,468	719			
Total	71	49,372				

Measurement 2

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	2,324	465	.55	2.37	3.34
Error	66	56,299	853			
Total	71	58,623				

Measurement 3

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	20,646	4,129	8.09**	2.37	3.34
Error	66	33,680	510			
Total	71	54,326				

** Significant at the 1% level of probability.

Measurement 4

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	9,248	1,850	4.07**	2.37	3.34
Error	66	29,997	454			
Total	71	39,245				

** Significant at the 1% level of probability.

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Table 98

Summary of Differences in Mean Salivary Performance Determined Immediately after Exposure to Ten Percent of Various Whole Smokes

Treatment	$\bar{x}$	Treatments				
		ADG Burley	ADF Bright	Mach. Exp.	Pall Mall	ABU TFP
1 (control)	76.4	45.7*	43.4	26.6	13.1	10.8
5 (ABU TFP)	65.6	34.9	32.6	15.8	2.3	
3 (Pall Mall)	63.3	32.6	30.3	13.5		
2 (mach. exp.)	49.8	19.1	16.8			
6 (ADF Bright)	33.0	2.3				
4 (ADG Burley)	30.7					

* Figure represents the difference between two means.
A difference of 26.7 or more is required for statistical significance at the 5% level.
Significant differences are underlined.

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Table 99

Summary of Differences in Mean Salivary Performances Determined One Hour after Exposure to Ten Percent of Various Whole Smokes

Treatment	$\bar{x}$	Treatments				
		ADF Bright	ADG Burley	Pall Mall	ABU TFP	Mach. Exp.
1 (control)	81.5	32.9*	25.8	13.3	9.2	6.5
2 (mach. exp.)	75.0	26.4	19.3	6.8	2.7	
5 (ABU TFP)	72.3	23.7	16.6	4.1		
3 (Pall Mall)	68.2	19.6	12.5			
4 (ADG Burley)	55.7	7.1				
6 (ADF Bright)	48.6					

* Figure represents the difference between two means.
A difference of 25.6 or more is required for statistical significance at the 5% level.
Significant differences are underlined.

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Table 100

The Effect of Gas Phases from Various Cigarettes on Salivary Performance of Male Inbred C57BL Mice (Smoke concentration ten percent)

Treatment	Measurements			
	1	2	3	4
1 (control)	83.7*	97.9	73.3	72.1
2 (machine exposed)	83.9	87.4	86.0	87.3
3 (Pall Mall)	77.6	90.6	76.5	84.9
4 (ADG Burley)	73.5	89.8	68.0	78.0
5 (ABU TFP)	80.3	86.0	60.6	84.7
6 (ADF Bright)	79.8	85.3	59.6	79.0

* Mean salivary performance of 12 mice.

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Table 101

Analysis of Variance

Measurement 1

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	920	184	.36	2.37	3.34
Error	66	33,554	508			
Total	71	34,474				

Measurement 2

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	1,281	256	.41	2.37	3.34
Error	66	41,403	627			
Total	71	42,684				

Measurement 3

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	6,089	1,218	1.34	2.37	3.34
Error	66	59,931	908			
Total	71	66,020				

Measurement 4

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	1,924	385	.80	2.37	3.34
Error	66	31,645	480			
Total	71	33,569				

1003112669

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 102

The Effect of Whole Smoke from Various Cigarettes on Salivary Performance of Male Inbred C57BL Mice (Smoke concentration 20 percent)

Treatment	Measurements			
	1	2	3	4
1 (control)	85.5*	76.9	97.3	94.6
2 (machine exposed)	88.2	74.2	86.8	102.5
3 (Pall Mall)	73.8	73.6	20.8	32.8
4 (ADG Burley)	82.4	79.4	7.8	13.2
5 (ABU TFP)	76.9	77.3	30.2	44.6
6 (ADF Bright)	81.5	67.3	11.9	13.9

* Mean salivary performance of 12 mice.

100312670

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 103

Analysis of Variance

Measurement 1

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	1,458	292	.36	2.37	3.34
Error	54	43,319	802			
Total	59	44,777				

Measurement 2

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	870	174	.20	2.37	3.34
Error	54	46,927	869			
Total	59	47,797				

Measurement 3

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	79,279	15,856	32.99**	2.37	3.34
Error	54	25,956	481			
Total	59	105,235				

** Significant at the 1% level of probability.

Measurement 4

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	78,340	15,668	48.30**	2.37	3.34
Error	54	17,512	324			
Total	59	95,852				

** Significant at the 1% level of probability.

10031126?1

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 104

Summary of Differences in Mean Salivary Performances Determined Immediately after Exposure to Twenty Percent of Various Whole Smokes

Treatment	$\bar{x}$	Treatments				
		ADG Burley	ADF Bright	Pall Mall	ABU TFP	Mach. Exp.
1 (control)	97.3	89.5*	85.4	76.5	67.1	10.5
2 (Mach. exp.)	86.8	79.0	74.9	66.0	56.6	
5 (ABU TFP)	30.2	76.5	22.4	9.4		
3 (Pall Mall)	20.8	13.0	8.9			
6 (ADF Bright)	11.9	4.1				
4 (ADG Burley)	7.8					

* Figure represents the difference between two means.
A difference of 25.8 or more is required for statistical significance at the 5% level.
Significant differences are underlined.

1003112672

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 105

Summary of Differences in Mean Salivary Performances Determined One Hour after Exposure to Twenty Percent of Various Whole Smokes

Treatment	$\bar{x}$	Treatments				
		ADG Burley	ADF Bright	Pall Mall	ABU TFP	Control
2 (mach. exp.)	102.5	89.3*	88.6	69.7	57.9	7.9
1 (control)	94.6	81.4	80.7	61.8	50.0	
5 (ABU TFP)	44.6	31.4	30.7	11.8		
3 (Pall Mall)	32.8	19.6	18.9			
6 (ADF Bright)	13.9	.7				
4 (ADG Burley)	13.2					

* Figure represents the difference between two means.
A difference of 20.9 or more is required for statistical significance at the 5% level.
Significant differences are underlined.

100312673

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 106

The Effect of Gas Phases from Various Cigarettes on Salivary Performance of Male Inbred C57BL Mice (Smoke concentration 20 percent)

Treatment	Measurements			
	1	2	3	4
1 (control)	75.9*	71.9	83.7	71.7
2 (machine exposed)	66.9	71.3	61.0	79.3
3 (Pall Mall)	60.8	79.4	43.5	66.0
4 (ADG Burley)	63.2	74.3	52.1	69.7
5 (ABU TFP)	66.9	68.8	27.8	52.2
6 (ADF Bright)	64.8	72.3	31.3	40.8

* Mean salivary performance of 12 mice.

1003112674

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 107

Analysis of Variance

Measurement 1

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	1,445	289	.38	2.37	3.34
Error	64	48,525	758			
Total	69	49,970				

Measurement 2

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	764	153	.18	2.37	3.34
Error	64	55,893	873			
Total	69	56,657				

Measurement 3

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	23,367	4,674	8.47**	2.37	3.34
Error	64	35,323	552			
Total	69	58,690				

** Significant at the 1% level of probability.

Measurement 4

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	11,904	2,381	3.08*	2.37	3.34
Error	64	49,501	774			
Total	69	61,405				

* Significant at the 5% level of probability.

100312675

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 108

Summary of Differences in Mean Salivary Performances Determined Immediately after Exposure to Twenty Percent of Various Gas Phases

Treatment	$\bar{x}$	Treatments				
		ABU TFP	ADF Bright	Pall Mall	ADG Burley	Mach. Exp.
1 (control)	83.7	55.9*	52.4	40.2	31.6	22.7
2 (mach. exp.)	61.0	33.2	29.7	17.5	8.9	
4 (ADG Burley)	52.1	24.3	20.8	8.6		
3 (Pall Mall)	43.5	15.7	12.2			
6 (ADF Bright)	31.3	3.5				
5 (ABU TFP)	27.8					

* Figure represents the difference between two means.
A difference of 28.1 or more is required for statistical significance at the 5% level.
Significant differences are underlined.

100312676

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 109

Summary of Differences in Mean Salivary Performances Determined One Hour after Exposure to Twenty Percent of Various Gas Phases

Treatment	$\bar{x}$	Treatments				
		ADF Bright	ABU TFP	Pall Mall	ADG Burley	Mach. Exp.
2 (mach. exp.)	79.3	38.5*	27.1	13.3		
1 (control)	71.7	30.9	19.5	5.7		
4 (ADG Burley)	69.7	28.9	17.5	3.7		
3 (Pall Mall)	66.0	25.2	13.8			
5 (ABU TFP)	52.2	11.4				
6 (ADF Bright)	40.8					

* Figure represents the difference between two means.
A difference of 33.3 or more is required for statistical significance at the 5% level.
Significant differences are underlined.

1003112677

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 110

The Effect of Whole Smoke from Various Cigarettes on Salivary Performance of Male Inbred C57BL Mice (Smoke concentration 30 percent)

Treatment	No. of Animals	Measurements			
		1	2	3	4
1 (control)	12	77.6	76.1	58.3	64.8
2 (machine exposed)	12	74.2	84.0	64.2	64.2
3 (Pall Mall)	0	-	-	-	-
4 (ADG Burley)	3	108.7	74.7	3.7	2.7
5 (ABU TFP)	8	86.3	88.6	6.4	8.5
6 (ADF Bright)	10	81.5	82.3	35.7	38.5

100312678

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 111

Analysis of Variance

Measurement 1

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	4	3,246	812	1.44	2.61	3.83
Error	40	22,515	563			
Total	44	25,761				

Measurement 2

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	4	1,061	265	.28	2.61	3.83
Error	40	38,470	962			
Total	44	39,531				

Measurement 3

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	4	33,793	8,448	19.10**	2.61	3.83
Error	40	17,723	443			
Total	44	51,516				

** Significant at the 1% level of probability.

Measurement 4

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	4	34,734	8,683	37.10**	2.61	3.83
Error	40	9,367	234			
Total	44	44,101				

** Significant at the 1% level of probability.

10031126 79

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 112

The Effect of Gas Phase from Various Cigarettes on Salivary Performance of Male Inbred C57BL Mice (Smoke concentration 30 percent)

Treatment	Measurements			
	1	2	3	4
1 (control)	74.6*	62.8	66.2	59.5
2 (machine exposed)	68.8	69.8	62.8	61.3
3 (Pall Mall)	67.4	70.2	36.6	64.4
4 (ADG Burley)	73.0	60.8	39.4	60.8
5 (ABU TFP)	68.9	68.1	35.4	54.3
6 (ADF Bright)	64.3	70.5	33.8	58.9

* Mean salivary performance of 12 mice.

1003112680

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 113

Analysis of Variance

Measurement 1

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	838	168	.30	2.37	3.34
Error	65	36,889	568			
Total	70	37,727				

Measurement 2

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	1,000	200	.28	2.37	3.34
Error	65	46,639	718			
Total	70	47,639				

Measurement 3

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	12,936	2,587	4.36**	2.37	3.34
Error	65	38,565	593			
Total	70	51,501				

** Significant at the 1% level of probability.

Measurement 4

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	664	133	.20	2.37	3.34
Error	65	42,464	653			
Total	70	43,128				

1003112681

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 114

Summary of Differences in Mean Salivary Performance Determined Immediately after Exposure to Thirty Percent of Various Gas Phases

Treatment	$\bar{x}$	Treatments				
		ADF Bright	ABU TFP	Pall Mall	ADG Burley	Mach. Exp.
1 (control)	66.2	32.4*	30.8	29.6	26.8	3.4
2 (mach. exp.)	62.8	29.0	27.4	26.2	23.4	
4 (ADG Burley)	39.4	5.6	4.0	2.8		
3 (Pall Mall)	36.6	2.8	1.2			
5 (ABU TFP)	35.4	1.6				
6 (ADF Bright)	33.8					

* Figure represents the difference between two means.
A difference of 30.7 or more is required for statistical significance at the 5% level.
Significant differences are underlined.

1003112682

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 115

Salivary Performance of Male C57BL Mice Immediately Following a Ten-Minute Exposure to 10, 20 or 30 Percent Whole Smoke or Gas Phase from Various Cigarettes (Expressed in mm of boundary displacement)

Whole Smoke

Treatments	Smoke Concentration (%)		
	10	20	30
Control	76.4 (12)	97.3 (12)	58.3 (12)
Machine exposed	49.8 (12)	86.8 (12)	64.2 (12)
ABU TFP	65.6 (12)	30.2 (12)	6.4 (8)
ADF Bright	33.0 (12)	11.9 (11)	35.7 (10)
ADG Burley	30.7 (12)	7.8 (5)	3.7 (3)
Pall Mall	63.3 (12)	20.8 (8)	- (0)

Gas Phase

Treatments	Smoke Concentration (%)		
	10	20	30
Control	73.3 (12)	83.7 (12)	66.2 (12)
Machine exposed	86.0 (12)	61.0 (12)	62.8 (12)
ABU TFP	60.6 (12)	27.8 (12)	35.4 (12)
ADF Bright	59.6 (12)	31.3 (12)	33.8 (12)
ADG Burley	68.0 (12)	52.1 (12)	39.4 (12)
Pall Mall	76.5 (12)	43.5 (12)	36.6 (12)

1003112683

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 116

Salivary Performance of Male C57BL Mice Immediately Following a Ten-Minute Exposure to 10, 20 or 30 Percent Whole Smoke or Gas Phase from Various Cigarettes

(Expressed in percentage from $\frac{\text{control + machine exposed}}{2}$)

Whole smoke

Treatment	Smoke Concentration (%)		
	10	20	30
$\frac{\text{Control + machine exposed}}{2}$	100	100	100
ABU TFP	104	33	10
ADF Bright	52	13	59
ADG Burley	49	8	6
Pall Mall	100	23	-
Pooled cigarette average	76	19	25

Gas phase

Treatment	Smoke Concentration (%)		
	10	20	30
$\frac{\text{Control + machine exposed}}{2}$	100	100	100
ABU TFP	76	38	55
ADF Bright	75	43	52
ADG Burley	85	72	61
Pall Mall	95	60	- 57
Pooled cigarette average	83	53	56

1003112684

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 117

Salivary Performance of Male C57BL Mice One Hour Following a Ten-Minute Exposure to 10, 20 or 30 Percent Whole Smoke of Gas Phase from Various Cigarettes
(Expressed in mm of boundary displacement)

Whole smoke

Treatment	Smoke Concentration (%)		
	10	20	30
Control	81.5 (12)	94.6 (12)	64.8 (12)
Machine exposed	75.0 (12)	102.5 (12)	64.2 (12)
ABU TFP	72.3 (12)	44.6 (12)	8.5 (8)
ADF Bright	48.6 (12)	13.9 (11)	38.5 (10)
ADG Burley	55.7 (12)	13.2 (5)	2.7 (3)
Pall Mall	68.2 (12)	32.8 (8)	--- (0)

Gas phase

Treatment	Smoke Concentration (%)		
	10	20	30
Control	72.1 (12)	71.7 (12)	59.5 (12)
Machine exposed	87.2 (12)	79.3 (12)	61.3 (12)
ABU TFP	84.7 (12)	52.2 (12)	54.3 (12)
ADF Bright	79.0 (12)	40.8 (12)	58.9 (12)
ADG Burley	78.0 (12)	69.7 (12)	60.8 (12)
Pall Mall	84.9 (12)	66.0 (12)	64.4 (12)

1003112685

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 118

Salivary Performance of Male C57BL Mice One Hour Following a Ten-Minute Exposure to 10, 20 or 30 Percent Whole Smoke or Gas Phase from Various Cigarettes

(Expressed in percentage from $\frac{\text{control + machine exposed}}{2}$)

Whole smoke

Treatment	Smoke Concentration (%)		
	10	20	30
$\frac{\text{Control+machine exposed}}{2}$	100	100	100
ABU TFP	92	45	13
ADF Bright	62	14	60
ADG Burley	71	13	4
Pall Mall	87	33	-
Pooled cigarette average	78	26	26

Gas phase

Treatment	Smoke Concentration (%)		
	10	20	30
$\frac{\text{Control+machine exposed}}{2}$	100	100	100
ABU TFP	106	69	90
ADF Bright	99	54	98
ADG Burley	98	92	101
Pall Mall	107	87	107
Pooled cigarette average	102	76	99

1003112686

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 119

Biological Characterization of 10, 20 or 30 Percent Whole Smoke and Gas Phase from ADG BURLEY Cigarettes

Whole smoke

Smoke Concentration	Mortality (%)	Salivary Performance (%)	
		Direct Effect	Aftereffect
10	0	49*	71*
20	58	8*	13*
30	75	6*	4*

Gas phase

Smoke Concentration	Mortality (%)	Salivary Performance (%)	
		Direct Effect	Aftereffect
10	0	85 NS	98 NS
20	0	72*	92 NS
30	0	61*	101 NS

* Significant at 5% level
NS Nonsignificant

1003112687

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 120

Biological Characterization of 10, 20 or 30 Percent Whole Smoke and Gas Phase from ADF BRIGHT Cigarettes

Whole smoke

Smoke Concentration	Mortality (%)	Salivary Performance (%)	
		Direct Effect	Aftereffect
10	0	52*	62*
20	8	13*	14*
30	17	59*	60*

Gas phase

Smoke Concentration	Mortality (%)	Salivary Performance (%)	
		Direct Effect	Aftereffect
10	0	75NS	99NS
20	0	43*	54*
30	0	52*	98NS

* Significant at 5% level
NS Nonsignificant

100312688

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 121

Biological Characterization of 10, 20 or 30 Percent Whole Smoke and Gas Phase from ABU TFP Cigarettes

Whole smoke

Smoke Concentration	Mortality (%)	Salivary Performance (%)	
		Direct Effect	Aftereffect
10	0	104 NS	92 NS
20	0	33 *	45 *
30	33	10 *	13 *

Gas phase

Smoke Concentration	Mortality (%)	Salivary Performance (%)	
		Direct Effect	Aftereffect
10	0	76 NS	106 NS
20	0	38 *	69 NS
30	0	55 *	90 NS

* Significant at 5% level
NS Nonsignificant

100312689

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 122

Biological Characterization of 10, 20 or 30 Percent Whole Smoke and Gas Phase from PALL MALL Cigarettes

Whole smoke

Smoke Concentration	Mortality (%)	Salivary Performance (%)	
		Direct Effect	Aftereffect
10	0	100^{NS}	87^{NS}
20	33	23*	33*
30	100	ND	ND

ND = not determined

Gas phase

Smoke Concentration	Mortality (%)	Salivary Performance (%)	
		Direct Effect	Aftereffect
10	0	95^{NS}	107^{NS}
20	0	60*	87^{NS}
30	0	57*	107^{NS}

* Significant at 5% level
NS Nonsignificant

1003112690

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 124

Mortality in Twenty-Four Male C57/BL and AKR/J Mice When Injected Subcutaneously with Various Doses of Nicotine Tartrate Immediately prior to the Third Salivation Measurement

C57BL/J

Treatments	No.	%
Control	0	0
Water	0	0
0.03 mg nicotine tartrate	0	0
0.10 mg nicotine tartrate	0	0
0.33 mg nicotine tartrate	0	0
1.00 mg nicotine tartrate	2	8

AKR/J

Treatments	No.	%
Control	0	0
Water	2	8
0.03 mg nicotine tartrate	2	8
0.10 mg nicotine tartrate	2	8
0.33 mg nicotine tartrate	4	17
1.00 mg nicotine tartrate	8	33

1003112691

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 125

Mean Salivary Performance of Male C57BL Mice When Injected Subcutaneously with Nicotine Tartrate Immediately Prior to Third Salivation

Treatment	No. of Animals	Measurement			
		1	2	3	4
Control	12	48.8	58.3	40.2	47.4
Water	12	48.8	50.0	40.0	37.4
0.03 mg nicotine tartrate	12	41.3	45.0	59.3	31.3
0.10 mg nicotine tartrate	12	54.1	40.6	57.0	42.0
0.30 mg nicotine tartrate	12	46.0	54.8	52.4	24.5
1.00 mg nicotine tartrate	12	42.3	39.5	52.3	24.5

10031112692

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 126

Analysis of Variance

Measurement 1

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	1,341	268	.27	2.37	3.34
Error	66	64,715	981			
Total	71	66,056				

Measurement 2

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	3,504	701	.79	2.37	3.34
Error	66	58,678	889			
Total	71	62,182				

Measurement 3

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	4,139	828	.87	2.37	3.34
Error	66	62,685	950			
Total	71	66,824				

Measurement 4

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	5,299	1,060	2.43*	2.37	3.34
Error	66	28,821	437			
Total	71	34,120				

* Significant at the 5% level of probability.

100312693

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 127

Summary of Differences in Mean Salivary Performance Determined One Hour after the Subcutaneous Injection of Various Dosages of Nicotine Tartrate in Male C57BL Mice

Treatment	$\bar{x}$	Treatments				
		Nicotine (1.0 mg)	Nicotine (0.3 mg)	Nicotine (0.03 mg)	Water	Nicotine (0.1 mg)
Control	47.4	22.9*	22.9	16.1	10.0	5.4
Nicotine (0.1 mg)	42.0	17.5	17.5	10.7	4.6	
Water	37.4	12.9	12.9	6.1		
Nicotine (0.03 mg)	31.3	6.8	6.8			
Nicotine (0.3 mg)	24.5	0.0				
Nicotine (1.0 mg)	24.5					

* Figure represents the difference between two means.
A difference of 25.1 or more is required for statistical significance at the 5% level.
Significant differences are underlined.

1003112694

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 128

Mean Salivary Performances of Male C57BL Mice When Injected Subcutaneously with Nicotine Tartrate Immediately Prior to Third Salivation

Treatment	No. of Animals	Measurement			
		1	2	3	4
Control	12	65.3	62.5	63.9	58.6
Water	12	67.0	59.0	69.9	63.9
0.03 mg nicotine tartrate	12	65.6	62.3	81.2	59.4
0.10 mg nicotine tartrate	12	59.4	70.6	32.0	57.7
0.30 mg nicotine tartrate	12	65.2	63.2	30.1	33.0
1.00 mg nicotine tartrate	10	64.5	65.9	32.6	32.0

100312695

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 129

Analysis of Variance

Measurement 1

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	413	83	.06	2.37	3.34
Error	64	82,331	1,286			
Total	69	82,744				

Measurement 2

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	922	184	.16	2.37	3.34
Error	64	72,758	1,137			
Total	69	73,680				

Measurement 3

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	30,090	6,018	6.14**	2.37	3.34
Error	64	62,747	980			
Total	69	92,837				

** Significant at the 1% level of probability.

Measurement 4

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	11,567	2,314	2.10	2.37	3.34
Error	64	70,436	1,1101			
Total	69	82,003				

100311269

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 130

Summary of Differences in Mean Salivary Performance Determined Immediately after the Subcutaneous Injection of Various Dosages of Nicotine Tartrate in Male C57BL Mice

Treatment	$\bar{x}$	Treatments				
		Nicotine (0.3 mg)	Nicotine (0.1 mg)	Nicotine (1.0 mg)	Control	Water
Nicotine (0.03 mg)	81.2	51.1*	49.2	48.6	17.3	11.3
Water	69.9	39.8	37.9	37.3	6.0	
Control	63.9	33.8	31.9	31.3		
Nicotine (1.0 mg)	32.6	2.5	0.6			
Nicotine (0.1 mg)	32.0	0.6				
Nicotine (0.3 mg)	30.1					

* Figure represents the difference between two means.
A difference of 37.6 or more is required for statistical significance at the 5% level.
Significant differences are underlined.

100312697

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 131

Mean Salivary Performance of Male AKR/J Mice When Injected Subcutaneously with Nicotine Tartrate Immediately Prior to Third Salivation

Treatment	No. of Animals	Measurement			
		1	2	3	4
Control	12	21.0	32.6	31.3	39.5
Water	10	27.8	27.6	34.7	38.7
0.03 mg nicotine tartrate	10	23.0	28.1	50.3	29.2
0.10 mg nicotine tartrate	10	18.2	31.8	39.6	19.8
0.30 mg nicotine tartrate	10	23.3	29.5	44.9	14.2
1.00 mg nicotine tartrate	6	26.0	33.5	41.2	20.0

100312698

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 132

Analysis of Variance

Measurement 1

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	564	113	.54	2.37	3.34
Error	52	10,879	209			
Total	57	11,443				

Measurement 2

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	274	55	.18	2.37	3.34
Error	52	15,584	300			
Total	57	15,858				

Measurement 3

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	2,504	501	1.12	2.37	3.34
Error	52	23,259	447			
Total	57	25,763				

Measurement 4

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	5,808	1,162	4.87**	2.37	3.34
Error	52	12,392	238			
Total	57	18,200				

** Significant at the 1% level of probability.

10031126 99

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 133

The Effect of Nitotine Tartrate Injected Immediately prior to Third Salivation on Salivary Performance of Male AKR/J Mice

Treatment	No. of Animals	Measurement			
		1	2	3	4
Control	12	20.5	29.8	24.3	15.5
Water	12	29.1	22.7	29.7	30.3
0.03 mg nicotine tartrate	12	27.8	23.2	35.7	18.7
0.10 mg nicotine tartrate	12	25.8	25.4	27.8	13.6
0.30 mg nicotine tartrate	10	21.6	23.7	32.3	7.8
1.00 mg nicotine tartrate	10	27.8	26.5	25.7	9.9

10031127700

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 134

Analysis of Variance

Measurement 1

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	726	145	.57	2.36	3.34
Error	62	15,925	257			
Total	67	16,651				

Measurement 2

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	424	85	.41	2.36	3.34
Error	62	12,790	206			
Total	67	13,214				

Measurement 3

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	1,042	208	.81	2.36	3.34
Error	62	15,954	257			
Total	67	16,996				

Measurement 4

Source	DF	SS	MS	F cal	F 95%	F 99%
Treatments	5	3,631	726	4.96**	2.36	3.34
Error	62	9,069	146			
Total	67	12,700				

** Significant at the 1% level of probability.

100312701

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 135

Summary of Differences in Mean Salivary Performance Determined One Hour after the Subcutaneous Injection of Various Dosages of Nicotine Tartrate

Treatment	$\bar{x}$	Treatments				
		Nicotine (0.3 mg)	Nicotine (1.0 mg)	Nicotine (0.1 mg)	Control	Nicotine (0.03 mg)
Distilled water	30.3	22.5*	20.4	16.7	14.8	11.6
Nicotine (0.03 mg)	18.7	10.9	8.8	5.1	3.2	
Control	15.5	7.7	5.6	1.9		
Nicotine (0.1 mg)	13.6	5.8	3.7			
Nicotine (1.0 mg)	9.9	2.1				
Nicotine (0.3 mg)	7.8					

* Figure represents the difference between two means.
A difference of 14.4 or more is required for statistical significance at the 5% level.
Significant differences are underlined.

10031127 02

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000

Table 136

Hypothesis on the Dual Responses by Nicotine Tartrate on Salivary Performance of Mice

Immediate Effect

Nicotine dose	High	Low
High salivation	↓	↑
Low salivation	↑	↑↑

Aftereffect
(One hour after injection)

Nicotine dose	High	Low
High salivation	↓	-
Low salivation	↓↓	↓

↓↓ strong inhibition
↓ inhibition
↑ stimulation
↑↑ strong stimulation
- no effect

100312703

Source: https://www.industrydocuments.ucsf.edu/docs/pgyl0000